Research Article

Higher Expression Level and Lower Toxicity of Genetically Spliced Rotavirus NSP4 in Comparison to the Full-Length Protein in *E. coli*

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Background: Rotavirus group A (RVA) is recognized as a major cause of severe gastroenteritis in children and new-born animals. Nonstructural protein 4 (NSP4) is responsible for the enterotoxic activity of these viruses in the villus epithelial cells. Amino acids 114-135 of NSP4 are known to form the diarrhea-inducing region of this viral enterotoxin. Therefore, developing an NSP4 lacking the enterotoxin domain could result in the introduction of a new subunit vaccine against rotaviruses in both humans and animals.
Objectives: The aim of this study is the evaluation of rotavirus A NSP4 expression in *E. coli* expression system before and after removal of the diarrhea-inducing domain, which is the first step towards further immunological studies of the resulting protein.
Materials and Methods: Splicing by overlap extension (SOEing) PCR was used to remove the diarrhea-inducing sequence from the NSP4 cDNA. Both the full-length (FL-NSP4) and the spliced (S-NSP4) cDNA amplicons were cloned into pET-32c and pGEX-6P-2. Expression levels of the recombinant proteins were evaluated in *E. coli* BL21 (DE3) by Western blot analysis. In addition, the toxicity of pET plasmids bearing the S-NSP4 and FL-NSP4 fragments was investigated by plasmid stability test.
Results: For FL-NSP4, protein expression was detected for the strain containing the pGEX:FL-NSP4 plasmid, but not for the strain carrying pET:FL-NSP4. Hourly sampling up to 3 h showed that the protein production decreased by time. In contrast, expression of S-NSP4 was detected for pET:S-NSP4 strain, but not for pGEX:S-NSP4. Plasmid stability test showed that pET:S-NSP4 recombinant plasmid was almost stable, while pET:FL-NSP4 was unstable.
Conclusions: This is the first report of production of rotavirus NSP4 lacking the diarrhea-inducing domain (S-NSP4). S-NSP4 shows less toxicity in this expression system and potentially could be a promising goal for rotavirus immunological and vaccine studies in the future.

Keywords: Diarrhea; Enterotoxin; Expression; NSP4; Rotavirus; Splicing by overlap extension PCR

1. Background

Rotaviruses are globally the leading cause of severe dehydrating diarrhea in children below 5 years of age. According to WHO estimates, rotaviruses resulted in approximately 453,000 (420,000-494,000) child deaths in 2008 worldwide, mostly in low income countries (1).

Rotaviruses belong to the genus *Rotavirus* in the family of *Reoviridae*. Rotavirus virions are non-enveloped forming an icosahedral triple-layered protein capsid the core of which carries the viral genome consisting of 11 segments of double-stranded RNA (dsRNA). The genome is surrounded by a triple-layered capsid and encodes six structural (VP1-VP4, VP6, and VP7) and six nonstructural (NSP1-NSP6) proteins. Nonstructural protein 4 (NSP4) is the enterotoxin of this virus, which plays its role in the villus epithelial cells and causes diarrhea (2).

The non-structural protein 4 (NSP4) is a 175-amino-acid enterotoxin with nascent polypeptide MW